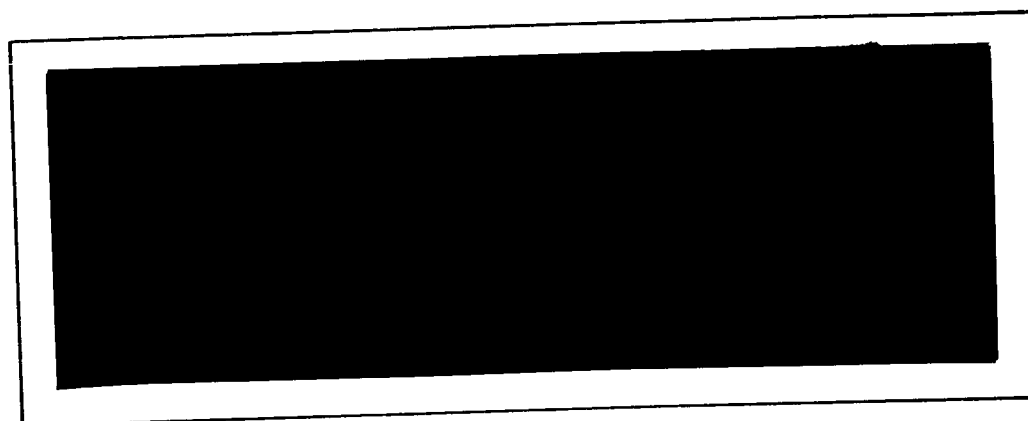


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Detection of Extraterrestrial Life.

Method II: Optical Rotatory Dispersion

Third Quarterly Report,

19 June 1963 to 20 September 1963

June 1963 43p (copy)

OTS

12

(NASA Contract No. NASw-557)

(NASA CR-52073) OTS: 44.60 p. 4, \$1.49-100

Office of Grants and Research Contracts, Code SC

National Aeronautics and Space Administration

Washington 25, D. C.

Submitted by

Melpar, Inc.

3000 Arlington Boulevard

Falls Church, Virginia

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ACKNOWLEDGMENT

The support of the National Aeronautics and Space Administration in the performance of work on Contract No. NASw-557 and this quarterly report is gratefully acknowledged.

TABLE OF CONTENTS

	<u>Page</u>
1.0 Introduction	5
2.0 Background	7
3.0 Experimental Procedures	10
4.0 Results	13
5.0 Conclusions	32
6.0 Summary	34
7.0 Program for Next Period	36
8.0 Appendix	37

LIST OF ILLUSTRATIONS

<u>Figure</u>		<u>Page</u>
1	Transmission of Polarizer and Analyzer with Angular Settings Between Their Optical Axis of (1) $+45^{\circ}$ and (2) -45°	14
2	Transmission of Polarizer and Analyzer with Their Optical Axis Parallel: (1) Percent Transmission of Polarizer and (2) Percent Transmission of Polarizer and Analyzer, Optical Axis Parallel	15
3	Transmission of Polarizer and Analyzer, Optical Axis Perpendicular	17
4	Optical Rotatory Dispersion Spectra of Sucrose: $+-$, 4.01 Degrees at Sodium D Line; $.-$, 2.01 Degrees at Sodium D Line; $\Delta - \Delta$, Literature Values	19
5	Optical Rotatory Dispersion Spectra of Tartaric Acid: (1) L-Tartaric Acid at 10% and (2) d-Tartaric Acid at 10%	21
6	Absorption Spectra of (1) Clear and (2) Cloudy Water-Soluble Extract of Soil (Concentration 0.65 mg/ml)	22
7	Optical Rotatory Dispersion Spectra of Clear and Cloudy Water-Soluble Extract of Soil (Concentration 0.65 mg/ml)	23
8	Absorption Spectra of Acid-Soluble Extract of Soil (Concentration 5.7 mg/ml)	25
9	Optical Rotatory Dispersion Spectra of Acid-Soluble Extract of Soil (Concentration 5.7 mg/ml)	26
10	Absorption Spectra of Base-Soluble Extracts of Soil: $0-0$, 0.15N NaOH Extract at 0.077 mg/ml and 0.5N NaOH Extract at 0.077 mg/ml	27
11	Optical Rotatory Dispersion Spectra of Base-Soluble Extracts of Soil (Concentration 0.077 mg/ml: (1) 0.15N NaOH Extract and (2) 0.5N NaOH Extract)	28
12	Optical Rotatory Dispersion Spectra of Adenosine (Concentration 0.021 mg/ml)	30
13	Optical Rotatory Dispersion Spectra of L-Tryptophan (Concentration 0.102 mg/ml)	31

1.0 INTRODUCTION

The object of this study is to develop polarimetric methods to detect the presence of optically active materials, particularly DNA (deoxyribonucleic acid) or its congeners in soil suspensions. Provided that such methods can be developed, the determination of the existence of life (as known terrestrially) on other planets may be accomplished by remote instrumentation. The cotton region of the spectrum associated with optically active biological compounds is of principal interest here because of the intensely high activity in the absorption bands of such materials.

In the previous report to NASA (Second Quarterly Report, 20 March 1963 to 19 June 1963), it was pointed out that certain problem areas have materialized in using the Rouy-type polarimeter. Results indicate that the measurements performed upon this instrument are qualitative rather than quantitative. These problem areas are as follows:

(a) The presence of reflecting surfaces which are located between the polarizer prisms and analyzer prisms result in the generation of elliptically polarized light. The amount of this light generated depends upon the angle with which the plane polarized beam makes with the reflecting surfaces. This elliptically polarized light can pass through the analyzer prism. Since the Rouy-type polarimeter contains two polarizing prisms, the positioning of the optic axis of these prisms with respect to the reflecting surfaces is critical. If the optic axes of the polarizing prisms are at different angles with respect to the reflecting surfaces, different amounts of elliptically polarized light

pass through the analyzer prism.

(b) The state of the art in preparing glan prisms has not achieved the state necessary for making precise optical rotatory measurements. The transmission of these prisms decreases rapidly at short wavelengths as a function of wavelength. In addition, the transmission of any two glan prisms at fixed wavelengths has proven impossible to match equally.

Theoretical consideration of these problems in the new ratio of electronic outputs, the difference to a constant, indicates that the measurement of optical rotatory dispersion should not be affected. The Cary model 15 spectrophotometer has been modified to perform these measurements. The polarimetric components for insertion into the sample and reference chamber of the spectrophotometer were illustrated in figure 3 and figure 4 in the second quarterly report to NASA (20 March 1963 to 19 June 1963).

Since the Rouy-type polarimeter at present is capable of giving only qualitative results, the effort during this last period has concentrated on the use of the new electronic ratio, the difference to a constant, in obtaining quantitative optical rotatory dispersion measurements of the optical activity of aqueous extracts of soil.

2. BACKGROUND

A complete theoretical development of the new electronic ratio, the difference to a constant was presented in the first quarterly report to NASA (20 December 1962 to 19 March 1963). This ratio can readily be measured on a double-beam instrument in which one beam contains both a polarizer and an analyzer prism, and the other beam contains only a polarizer prism. By setting the optical axis of the analyzer at an angle of $(+\theta)$ and then at $(-\theta)$ with respect to the optical axis of the polarizer, optical rotation in the presence of absorption, scattering and circular dichroism may be determined. The key to this method is that one beam of plane polarized light is directed through a sample and allowed to fall on an analyzer prism. The light which passes through the analyzer then impinges on a photomultiplier tube. The other beam following a path containing only the polarizing prism and sample also impinges on a matched photomultiplier tube. The ratio of the currents which are produced by the photomultiplier tubes results in a function which is linear with respect to the optical activity of the sample. The relationship which expresses the ratio of the difference to a constant, in terms of optical activity, is represented by equation (1).

$$R = 4t\alpha \left[1 - \frac{(\Delta Klc)^2}{8} \right] \quad (1)$$

where

R = the ratio of the difference to a constant

α = angular rotation of the sample in radians

$t = \tan \theta$, where θ is the angle between the optical axis of

the analyzer and the optical axis of the polarizer
 ΔK = difference in absorption coefficients associated with
circular dichroism, the unequal absorption of right
and left circularly polarized light by an optically
active sample.

l = path length

c = concentration of the optically active solution

In the second quarterly report to NASA (20 March 1963 to 19 June 1963), the problem of unequal transmission of the glan polarizing prism at specified wavelengths was considered in this ratio; it was shown that in contrast to the Rouy approach, the measurement of optical rotation should/not be effected by this effect.

In addition, optical activity can also be measured by using a simple ratio in which the optical axis of the analyzer is fixed at an angle of either $+45$ or -45 degrees with respect to the optical axis of the polarizer. This approach also permits the measurement of the optical activity of a sample which exhibits absorption, scattering, and circular dichroism. The theoretical development of this method is given in the Appendix. The simple ratio, R' , (see Appendix) may be expressed in terms of angular rotation (α) and the dichroic effect (ΔK) by equation (2) or equation (3), depending upon whether the angle between the optical axis of the analyzer and polarizer is set when at $+45$ or -45 degrees, respectively,

$$R' = 1 + 2t\alpha \left[1 - \frac{(\Delta Klc)^2}{8} \right] \quad (2)$$

$$R' = 1 - 2t\alpha \left[1 - \frac{(\Delta Klc)^2}{8} \right] \quad (3)$$

Consideration of the unequal transmission of the glan polarizing prisms here indicated that it should not effect the measurement of optical rotation by this method either.

3. EXPERIMENTAL PROCEDURES

All rotatory dispersion measurements were performed on the Cary, model 15, spectrophotometer containing the specially designed inserts which were described in the second quarterly report to NASA (20 March 1963 to 19 June 1963). The optical components which make up these inserts were aligned on an optical bench. The angular settings of +45 and -45 degrees between the optical axis of the analyzer and polarizer were determined by measurement at the sodium D line on the Rudolph polarimeter, model 70. It was necessary to remove the half-shade prism and the polarizer to perform this operation. The sample insert containing only the polarizing prism was aligned in the Rudolph polarimeter. The plane of polarized light emitted from this prism was determined by the rotation of the analyzer prism located within this polarimeter. The analyzer prism in the Rudolph polarimeter was then either rotated +45 or -45 degrees from this point. The polarizing prism contained within the sample insert was removed and replaced by the analyzer prism. This prism was then rotated until a null was achieved. Thus, the angle between the optical axis of the analyzer and polarizer could be set either at +45 or -45 degrees.

Water, 0.15 N HCl, 0.15 N NaOH, and 0.5 N NaOH extracts of soils, obtained in the vicinity of Melpar, laboratories were prepared in the following manner. 200 g of dried soil was treated with 137.5 ml of water, and resulting mixture stirred for two hours. This was found to be sufficient time for resaturation of the supernatant to occur. The water-soluble extract was then separated from the solid materials by centrifuging in an International centrifuge. The supernatant was decanted from the solid material and filtered through a 0.45-micron millipore filter.

The volume of supernatant collected was approximately 60 ml. The concentration of dissolved components within the water soluble extract was about 0.65 mg/ml.

The acid-soluble extract of soil was prepared by adding 55 ml of 0.15 N HCl to 50 g of dried soil. This mixture was stirred for 18 hours to ensure saturation of the supernatant. Separation of the supernatant from the solid material was as described for the preparation of the water-soluble extract. The volume of acid-soluble extract collected was 35 ml. The concentration of dissolved components in this extract was found to be approximately 11.4 mg/ml.

Both the 0.15 NaOH and 0.5N NaOH extract were prepared in a manner similar to the acid extract. Because of the nature of these extracts, a Spinco, model L, preparative ultracentrifuge was necessary to separate the extract initially from the solid material. This step required a speed of 40,000 rpm for 1 hour. A 0.45-micron millipore filter was also used for the final separation. The volume of both extracts collected was around 33.8 ml. The concentration of dissolved components in these extracts was found to be 7.7 mg/ml.

An indication of the extraction efficiencies of the various solvents is received by noting that the optical measurements were performed on the water-soluble extract without any dilution. The acid-soluble extract required a dilution of 1:2 and a dilution of 1:100 was required for the base soluble extracts.

All optical rotatory dispersion measurements were repeated a minimum of three times. These repeated measurements were necessary because of a drift in the base line which was caused by using the Cary spectrophotometer as a polarimeter.

Reagent-grade sucrose used in these experiments was obtained from Fischer Scientific Co. D and L tartaric acid, adenosine and L tryptophan, all of A grade classification, were obtained from Cal Biochem.

4.0 RESULTS

Although the theoretical development of the electronic ratio, the difference to a constant, or the simple ratio, indicated that polarimetric measurement could be made on optically active substances in the cotton region of the spectrum, experimentation was necessary to prove the validity of these theoretical considerations.

To be able to apply both the ratio, the difference to a constant, and the simple ratio, the angle between the optical axis of the analyzer and polarizer had to be set at $+45$ and the -45 degrees. Measurements indicated that the transmission of the Glan analyzer prism was not the same at each of these settings. These results are shown in figure 1. Although the Spectral absorptions of both prisms are superimposable for both settings, the angular setting for $+45$ degrees had a transmission of about 3 per cent greater than that of the -45 degrees over the spectral range examined.

This result lead us to concentrate on the simple ratio, since the simple ratio requires only one angular setting of either $+45$ degrees or -45 degrees between optical axis of the analyzer and polarizer, in contrast to the use of the difference to a constant ratio, which requires the use of both angular settings. In addition, for one given angular setting between the optical axis of the analyzer and polarizer, the absorption term (e^{-kc}) associated with the absorption of plane-polarized light by the analyzer prism cancels when considered in E' and E of equation 1 (See appendix). Evidence of this absorption is show in figure 2, where a comparison is made between the transmission of the polarizing

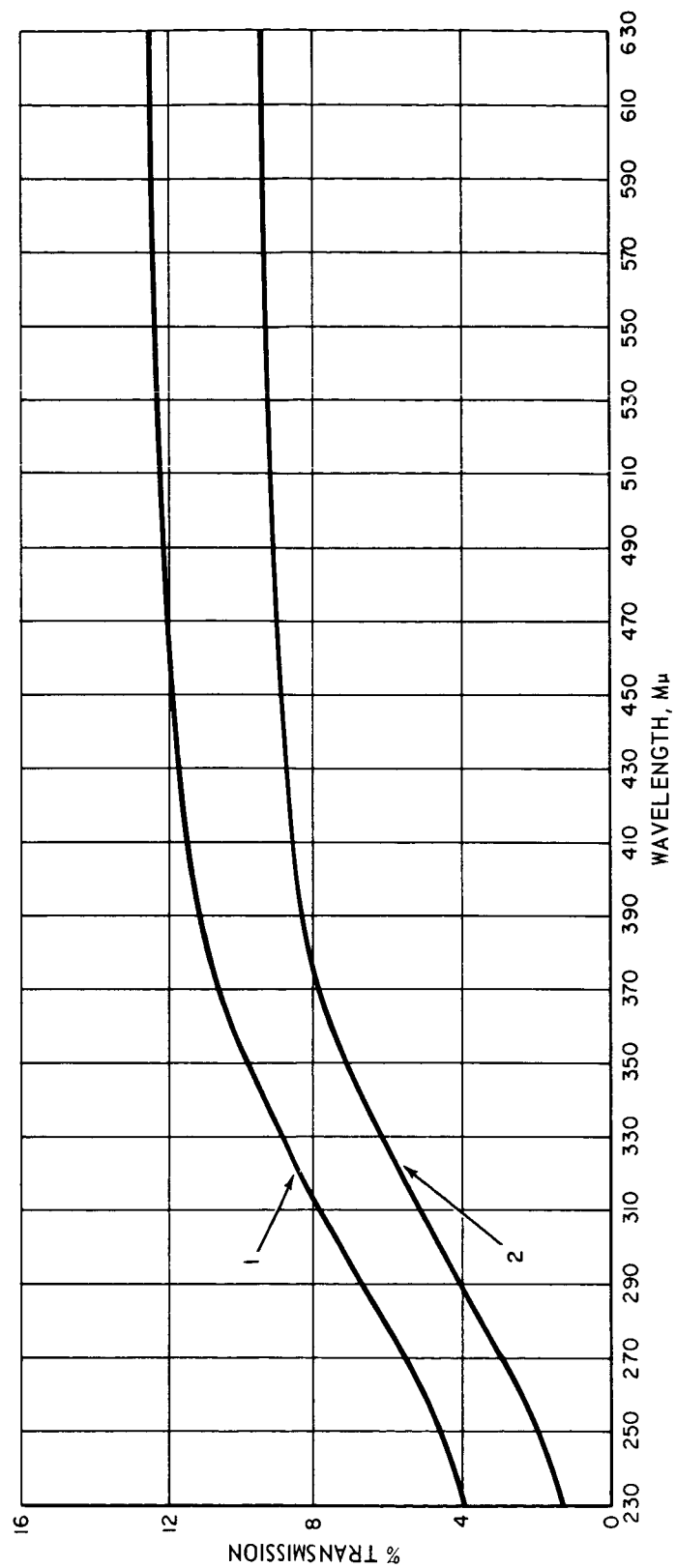


Figure 1. Transmission of Polarizer and Analyzer with Angular Settings Between Their Optical Axis of (1) $+45^\circ$ and (2) -45°

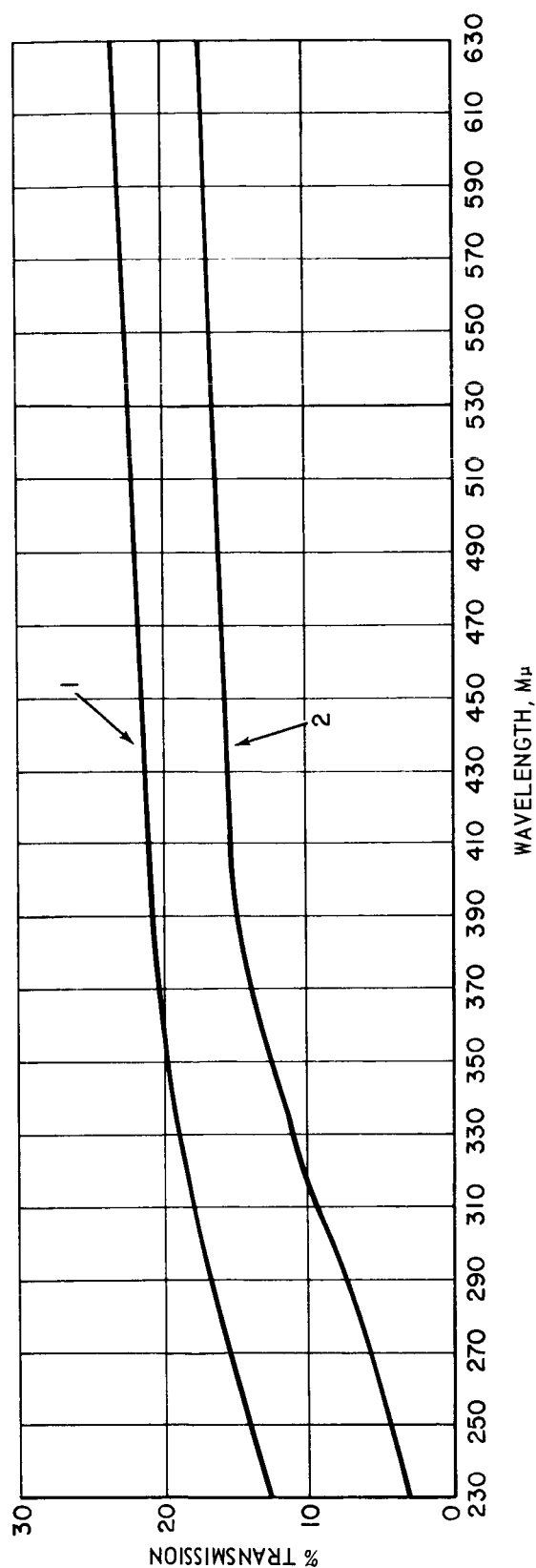


Figure 2. Transmission of Polarizer and Analyzer with Their Optical Axis Parallel: (1) Percent Transmission of Polarizer and (2) Percent Transmission of Polarizer and Analyzer, Optical Axis Parallel

prism and the transmission of both the polarizing and analyzer prisms for which their optical axes are parallel.

It was also desirable to determine whether all of the light passing through the polarizing prism was plane polarized. This was accomplished by setting the optical axes of the polarizer and analyzer perpendicular to one another. The results of these measurements are shown in figure 3. The maximum amount of unpolarized light passing through the analyzer prism occurs at about 585 mμ. The percent transmission is about 1.7×10^{-2} . This indicates that only 0.14% of the plane-polarized beam is unpolarized because the total percent transmission at this wavelength for an angular setting of 45 degrees between the optical axis of the analyzer and polarizer is 12.3.

To test the validity of measuring optical rotation by the simple ratio as represented by equation (2) and equation (3) (See section 2.0), the optical activity of sucrose was measured in the wavelength range from 650 mμ to 290 mμ. For this series of measurements, the angle between the optical axis of the analyzer was set at +45 degrees to obtain as much transmission of plane-polarized light through the prisms as possible. For this angular setting, the relationship between the simple ratio and optical activity is represented by equation (2). Because sucrose exhibits no absorption peak in this wavelength region, the dichroic term (ΔK) in this equation can be considered negligible. The results of these measurements for two sucrose solutions which were prepared to give a two-degree and four-degree rotation at the sodium D line are shown in

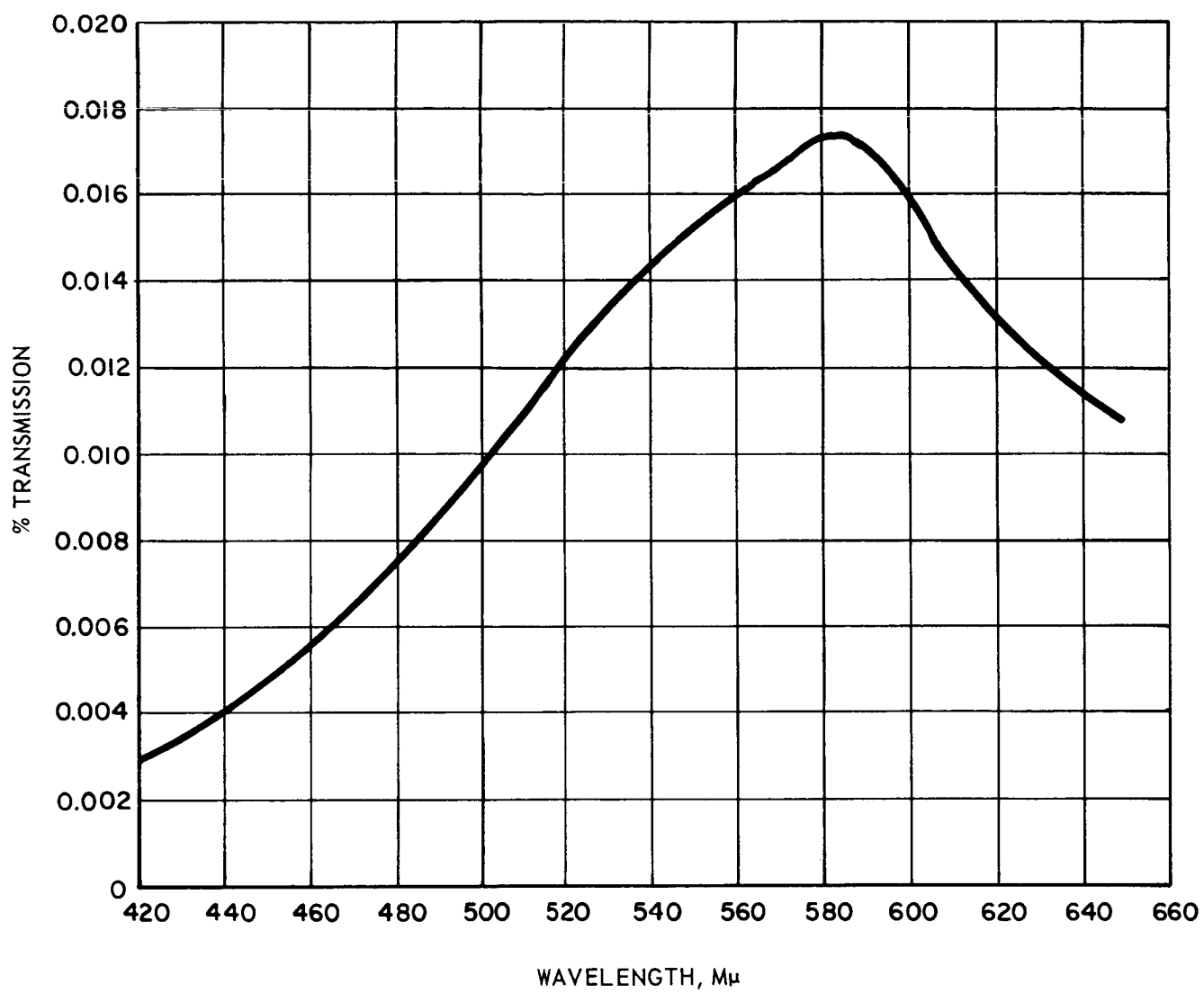


Figure 3. Transmission of Polarizer and Analyzer, Optical Axis Perpendicular

figure 4. The triangular points represent the angular rotations calculated from the specific rotations of sucrose obtained from the literature (Handbook of Chemistry and Physics, 35th Edition, Chemical Rubber Publishing Co., p. 2734). Figure 4 shows that the angular rotation calculated from the literature is in agreement with those obtained by measuring the simple ratio. The reproducibility of these measurements is within 0.5 degrees. Theoretical considerations, however, indicate that the Cary, model 15, spectrophotometer should give agreement within 0.1 degrees at an angular setting of +45 degrees between the optical axes of the analyzer and polarizer. The reason for this discrepancy appears to be a drift in the instrumental base line. The positioning of a polarizing prism in the reference beam of this spectrophotometer causes this drift because the automatic slit control depends upon the transmission of the reference beam. The prism in one reference beam absorbs strongly, the slit opens to balance the circuit, and above a slit width of 0.25 mm there appears to be poor reproducibility. In the ultraviolet region of the spectrum, this shift is even more pronounced because the transmission of the Glan prisms is below 10 percent. Based upon the finding above, the Cary spectrophotometer was used as a single-beam instrument for solutions with an optical density above 0.5. In other words, each individual transmission (E) which is designated by equation (1) (see appendix) was measured separately.

From equation (2) or equation (3) (See Section 2), the simple ratio (R'), plotted as a function of angular rotations (α), should be symmetrical about 1 when the angle between the optical axis of the

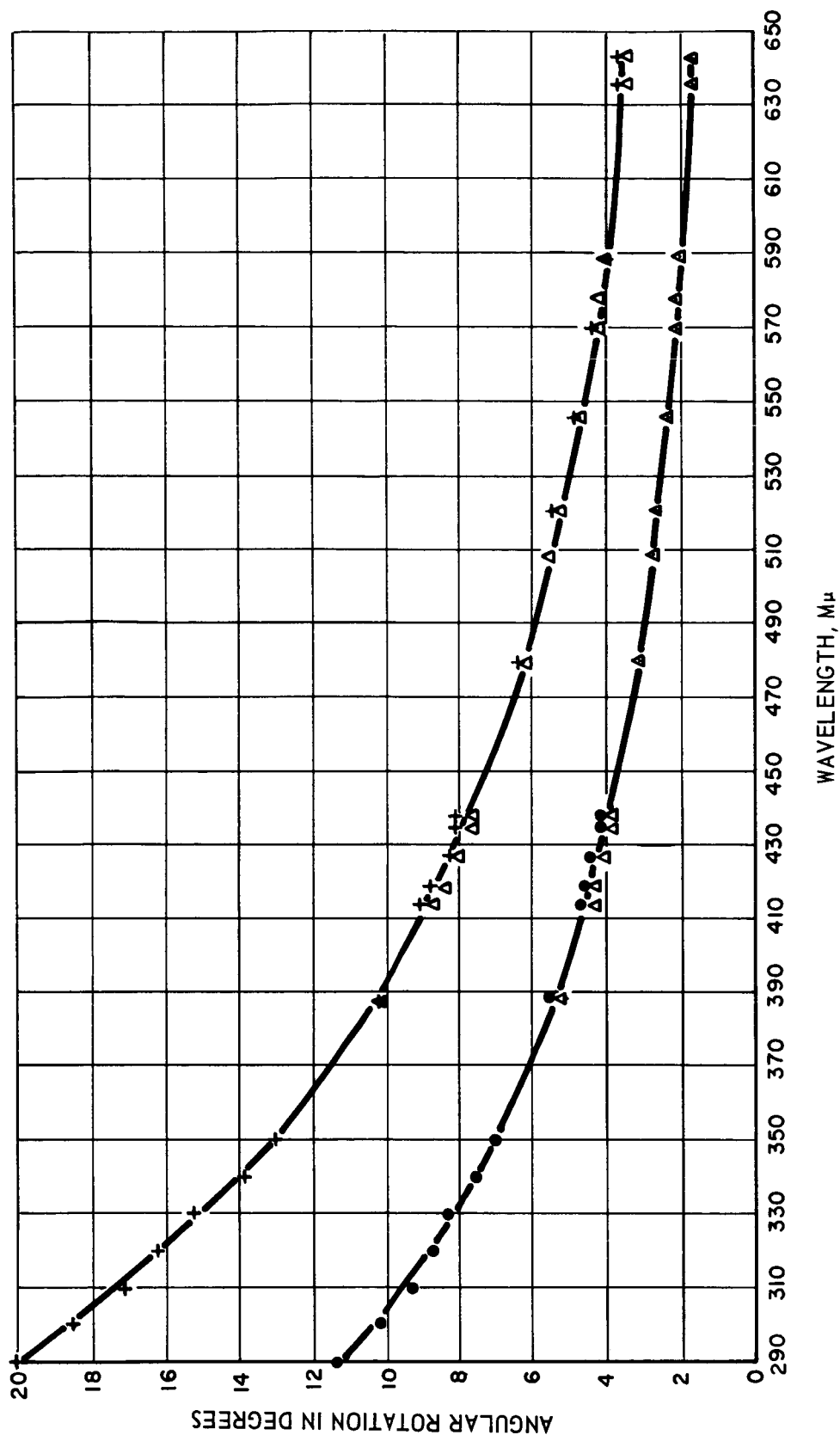


Figure 4. Optical Rotatory Dispersion Spectra of Sucrose: + - +, 4.01 Degrees at Sodium D Line; . - ., 2.01 Degrees at Sodium D Line; Δ - Δ , Literature Values

analyzer and polarizer is set at either +45 or -45 degrees. This is because the $\cos 2\alpha$ term in equation (7) (See appendix) becomes zero. Experimentally, this may be shown to be valid in the following manner. The angular rotation of the D and L form of tartaric acid should be of the same magnitude, but different in sign. The optical rotatory dispersion curves for 10% solutions of D and L tartaric acid for the wavelength range from 350 to 250 $m\mu$ are shown in figure 5. For a given wavelength, the angular rotation for both forms of tartaric acid are the same, but opposite in sign.

In the theoretical development of the simple ratio, an assumption that the scattering term (Σ) in equation (4a) or equation (4b) (see appendix) may be neglected because this quantity is small compared to the total intensity as seen by the photomultiplier tube. There is some experimental evidence to indicate that this assumption is valid. The water-soluble extract of soil, when freshly prepared, is clear; upon standing overnight, however, it becomes cloudy. Absorption measurements upon the clear and cloudy extract indicate both curves to have the same shape but with a difference in optical density. Figure 6 shows these absorption curves. Based upon these observations, it appears that finely divided particles of silica precipitate and scatter light. Optical rotatory measurements by the simple ratio indicate no differences in angular rotation between these solutions. The results of these measurements are shown in figure 7. Thus, it appears that the presence of particles capable of scattering light have no effect on the measurement of optical activity by the simple ratio technique.

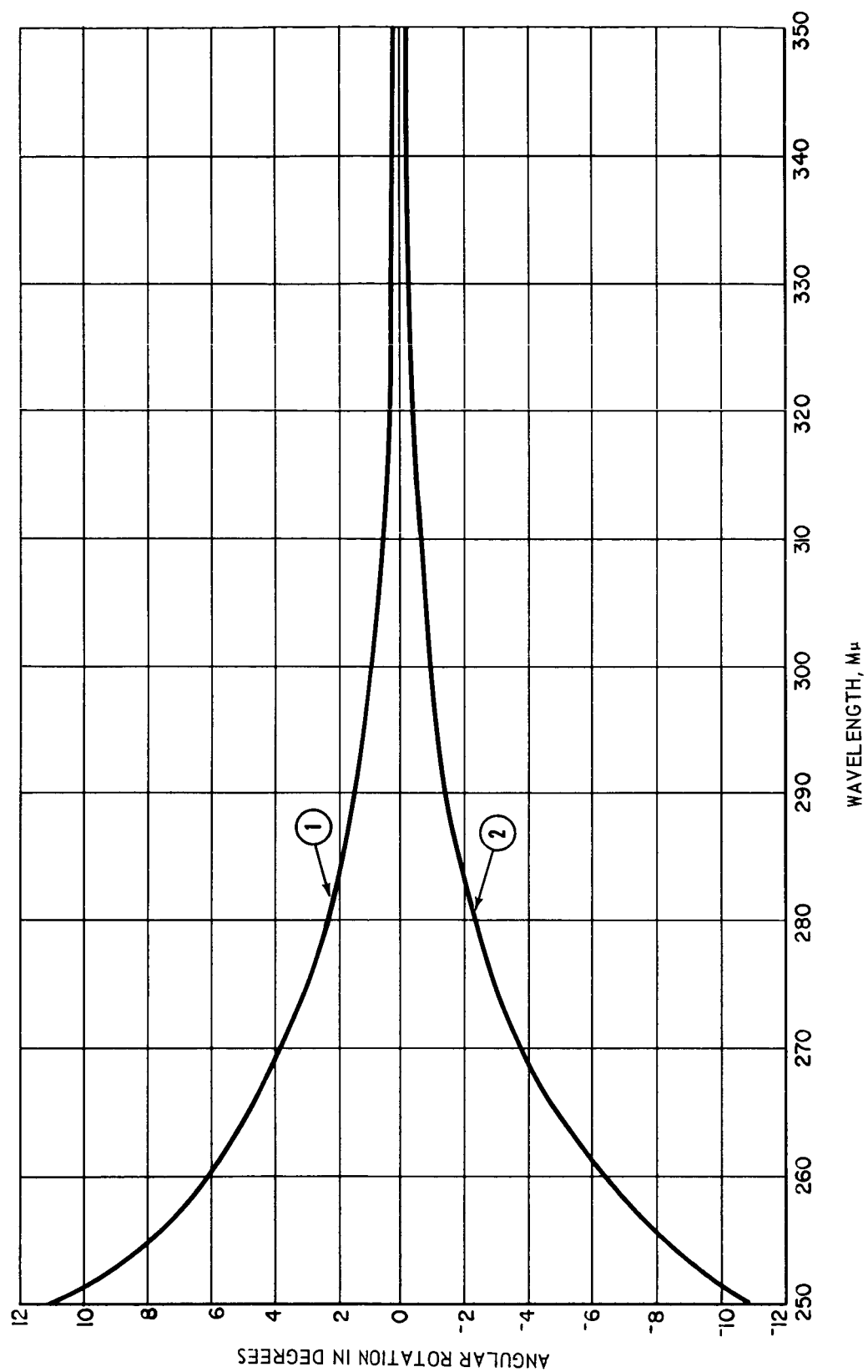


Figure 5. Optical Rotatory Dispersion Spectra of Tartaric Acid: (1) L-Tartaric Acid at 10% and (2) d-Tartaric Acid at 10%

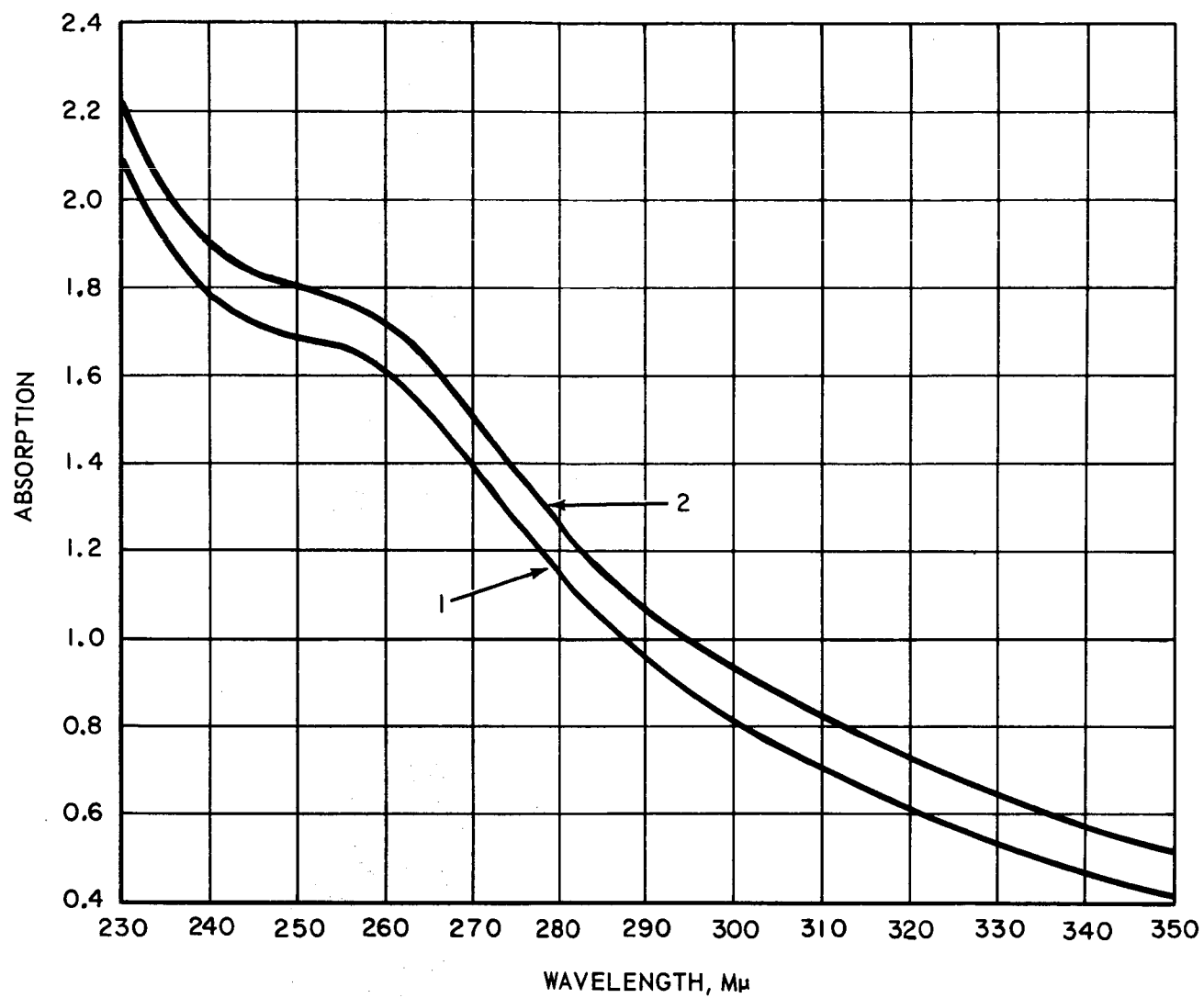


Figure 6. Absorption Spectra of (1) Clear and (2) Cloudy Water-Soluble Extract of Soil (Concentration 0.65 mg/ml)

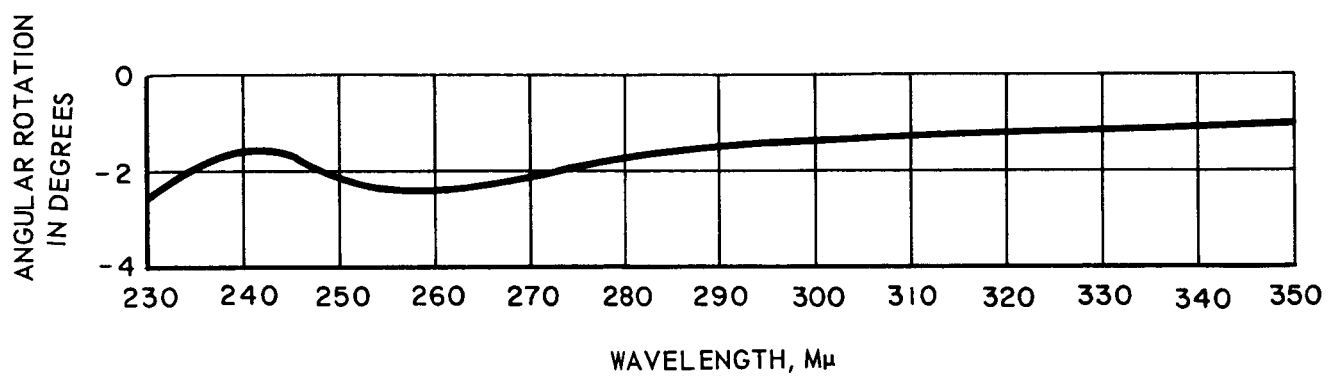


Figure 7. Optical Rotatory Dispersion Spectra of Clear and Cloudy Water-Soluble Extract of Soil (Concentration 0.65 mg/ml)

Further examination of figure 6 reveals an absorption peak at approximately 250 m μ for both the clear and cloudy extract. From figure 7, there appears to be a cotton effect associated with this absorption peak with a minimum at 255 m μ and a maximum at approximately 244 m μ . The specific rotation at this minimum is calculated to be approximately $-3.7 \times 10^{+4}$ degrees.

Figures 8 and 9 show the absorption and the optical rotatory dispersion curve, respectively, for the acid extract of soil. Comparison of the curves in these two figures with figure 7 indicates that the absorption peak is still present at 250 m μ , whereas the cotton effect is no longer apparent. It appeared that substances present in the water-soluble extract were hydrolyzed in acid solution. To substantiate this conclusion further, the specific rotation for the acid-soluble extract at 255 m μ was calculated to be approximately -4.7×10^3 degrees. This is a considerable decrease in specific rotation when compared to that of the water-soluble extract, -3.7×10^4 degrees.

Although the acid-soluble extract has a lower specific rotation than the water-soluble extract, the components in the soil are far more soluble in acid solution. The concentration of a saturated solution of the acid-soluble components was found to be 11.4 mg/ml, whereas a saturated solution of the water-soluble components was only 0.65 mg/ml.

The absorption and optical rotatory dispersion curves for the base-soluble extract of soil are presented in figures 10 and 11, respectively. Separate samples of soil were treated with 0.15 N NaOH and 0.5 N NaOH solutions. Because any RNA and DNA present in the soil are normally

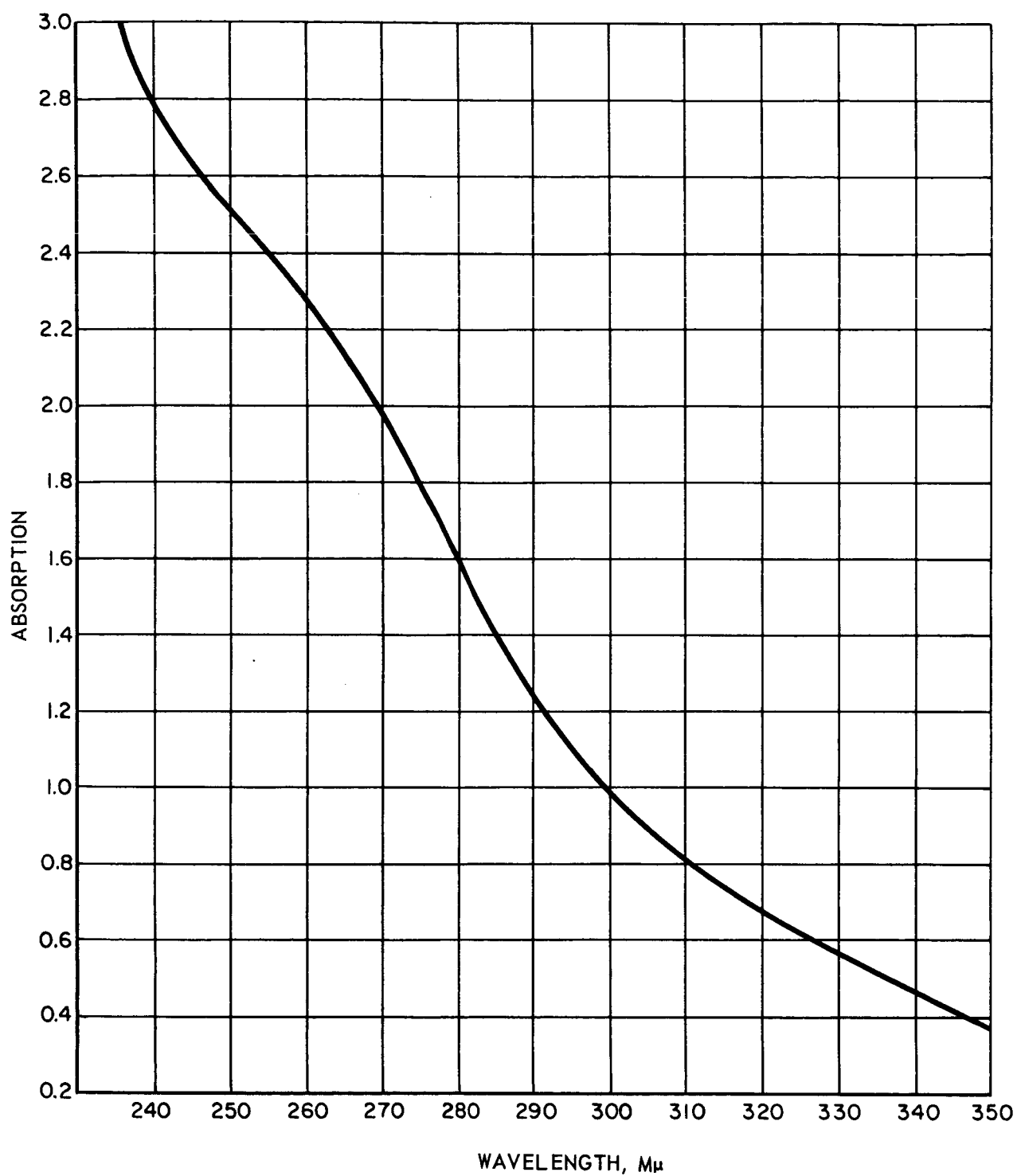


Figure 8. Absorption Spectra of Acid-Soluble Extract of Soil (Concentration 5.7 mg/ml)

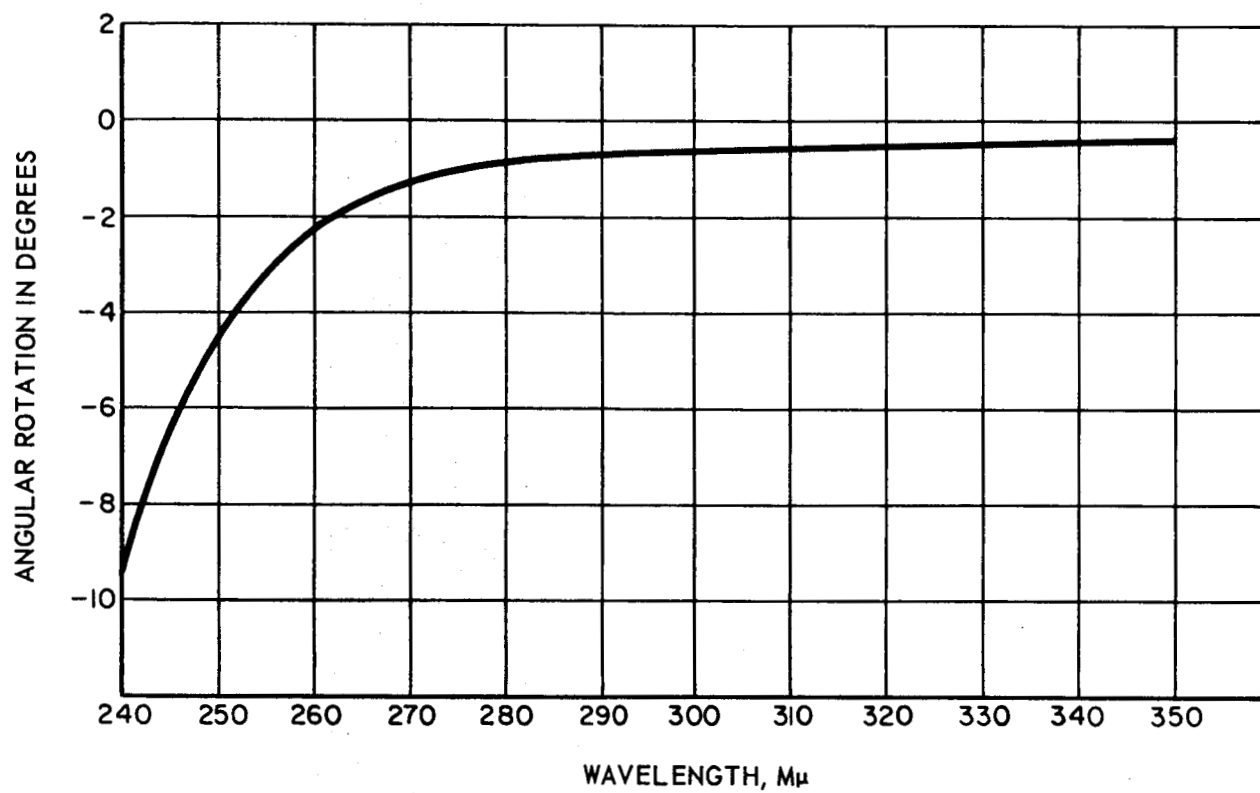


Figure 9. Optical Rotatory Dispersion Spectra of Acid-Soluble Extract of Soil (Concentration 5.7 mg/ml)

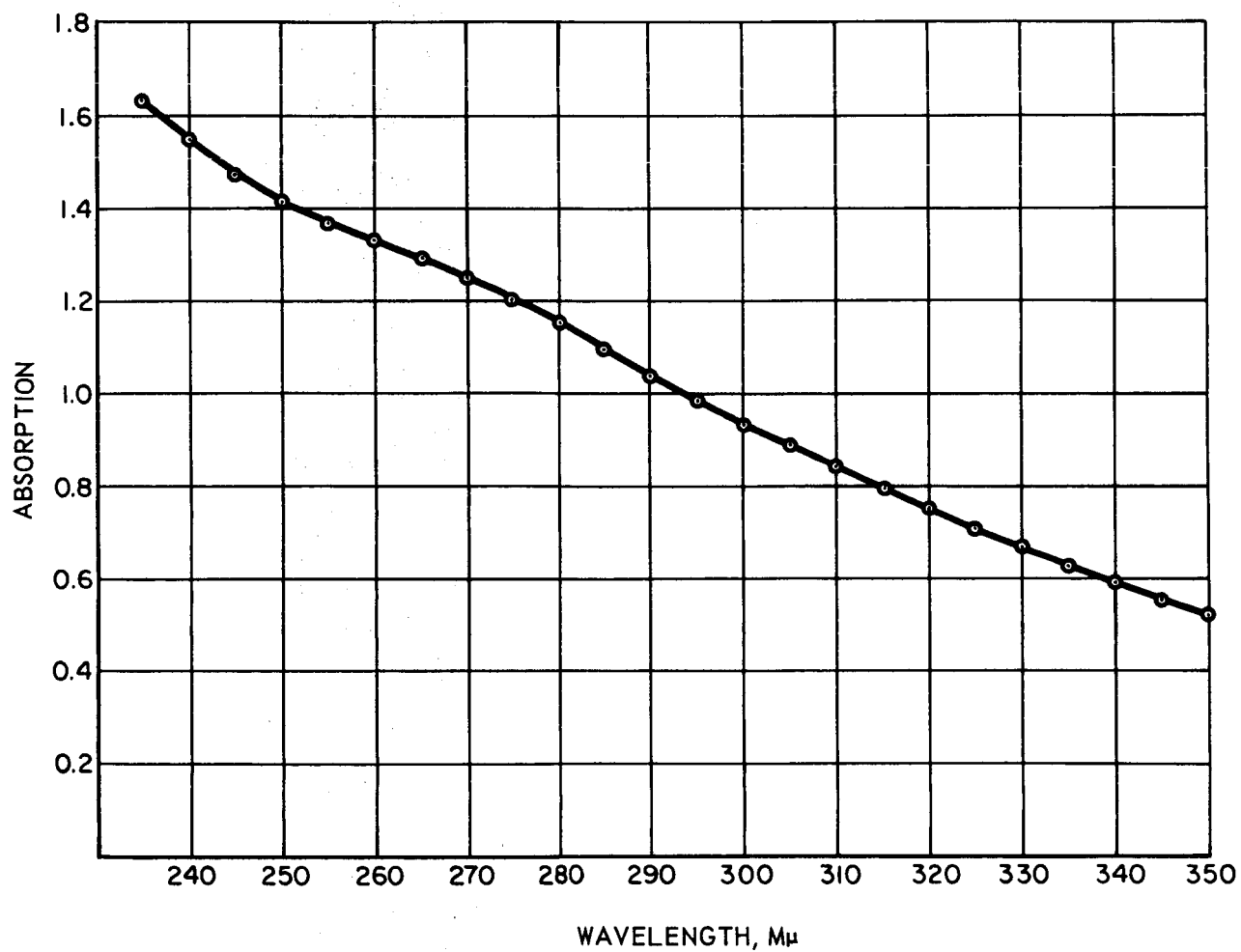


Figure 10. Absorption Spectra of Base-Soluble Extracts of Soil: 0 - 0, 0.15N NaOH Extract at 0.077 mg/ml and, 0.5N NaOH Extract at 0.077 mg/ml

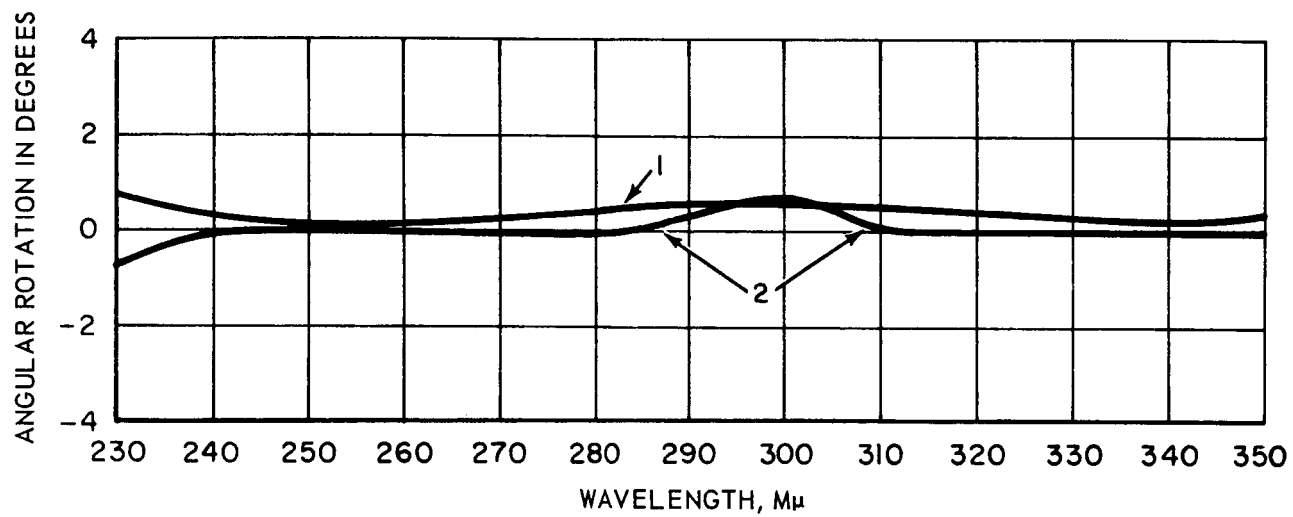


Figure 11. Optical Rotatory Dispersion Spectra of Base-Soluble Extracts of Soil (Concentration 0.077 mg/ml: (1) 0.15N NaOH Extract and (2) 0.5N NaOH Extract)

found in the humic acid fraction or base-soluble extract of soil (G. Anderson, Soil Science, 48, 169 (1958), it was expected that, by using the more basic solution, any RNA present in the soil would hydrolyzed. Thus, the angular rotations for 0.5 N NaOH extract would be more positive than for the 0.15 N NaOH extract. This is based upon the fact that the dispersion curve for adenosine in figure 12 indicates positive rotations in the wavelength region from 260 to 230 m μ . The more basic extract, however, does not appear to have any measurable rotation in this wavelength region except below 240 m μ , where it exhibits negative rotations. It appears as if the more basic solution is capable of destroying the optical activity. A comparison of the absorption curves for both extracts in figure 10 indicates that they are superimposable, suggesting that the same chromophores are still present in both extracts.

It may be that negative rotations observed for the acid-soluble and the 0.5 N NaOH extract could be caused by the presence of proteins or its hydrolysis products, since the rotatory dispersion course of L-tyrphophane (figure 13) a representative protein component, shows strong negative rotation.

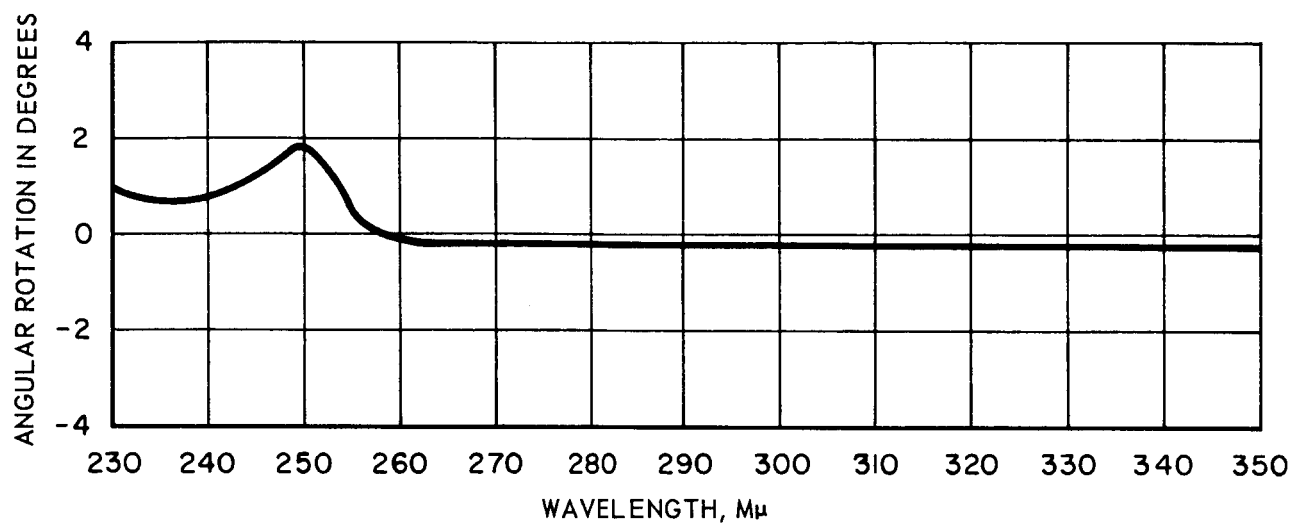


Figure 12. Optical Rotatory Dispersion Spectra of Adenosine (Concentration 0.021 mg/ml)

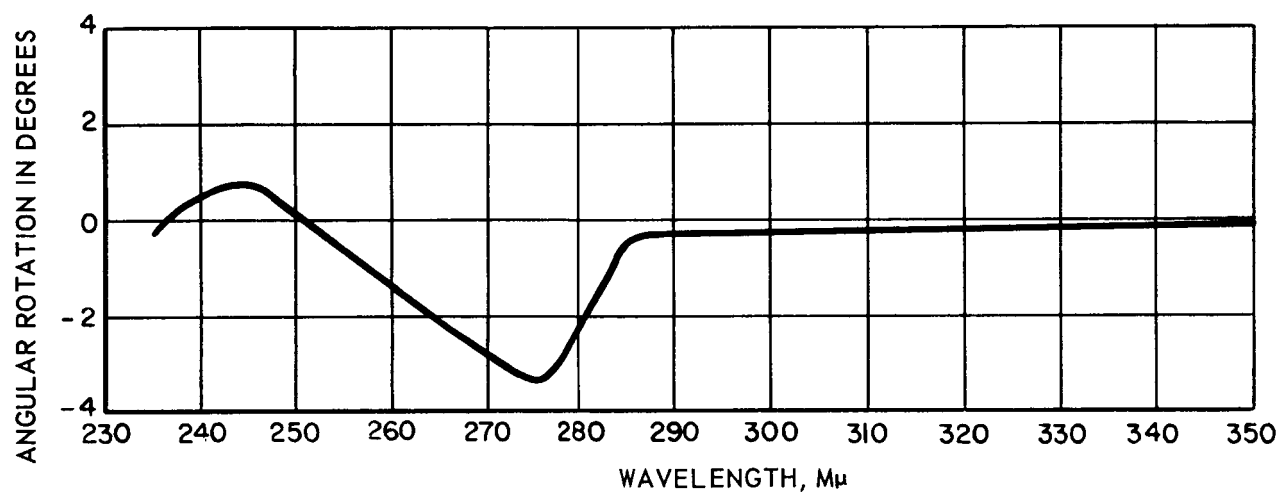


Figure 13. Optical Rotatory Dispersion Spectra of L-Tryptophan (Concentration 0.102 mg/ml)

5.0 CONCLUSIONS

The data presented indicates that the theoretical development of the electronic simple ratio for obtaining optical rotatory dispersion measurements is valid. Common instrumental problem areas, such as the unequal transmission of the glan polarizing prisms, which render the Rouy-type polarimeter a qualitative instrument, do not appear to affect the optical rotation measurements by the simple ratio technique.

The use of the Cary, model 15, spectrophotometer as a polarimeter can introduce errors as large as 0.5 degrees into the measurement of optical rotations because of the automatic slit control. These errors are the result of a drift in the base line. Several measurements on the same sample, however have reduced this uncertainty to about 0.1 degrees.

The results of the optical rotation measurements on water, acid, and basic soluble extracts of soil indicate that the specific rotation at 255 m μ for optically active components in the 0.15N HCl and 0.5N NaOH are lower than the rotations found for the water-soluble and 0.15N NaOH extracts.

A comparison of the rotatory dispersion curves for both basic extracts shows that they are different in the wavelength region from 280 to 230 m μ . The more basic extract does not appear to have any measurable rotation, even though the absorption curves for both extracts are superimposable. This evidence suggests that the use of the more basic extraction process results in hydrolysis of chemical bonds responsible for the optical activity of the 0.15N NaOH extract.

The positive rotations observed for 0.15N extract in the wavelength region from 260 to 230 $m\mu$ indicate the possible presence of nucleic acids or their derivatives. This is based upon the fact that adenosine in this wavelength region also exhibits positive rotations.

6.0 SUMMARY

An apparatus has been designed and incorporated into the Cary, model 15, spectrophotometer which permits premeasurement of optical rotation in media which exhibit absorption, scattering and circular dichroism. The theoretical development of this approach is presented. The optical rotatory dispersion measurements performed on solutions of sucrose, D and L tartaric acid, and water-soluble extract of soil have established the validity of this development.

Optical rotatory dispersion measurements have also been obtained on pure water, 0.15N HCl, 0.15N NaOH and 0.5N NaOH soluble extracts of soil. In the wavelength region from 230 to 280 m μ both the water and acid-soluble extracts of soil exhibit negative optical rotations. The 0.15N NaOH extract of soil shows a positive optical rotation in this wavelength region. The 0.5N NaOH extract indicates no measurable rotation in this wavelength region. Below 240 m μ , however, the more basic extract negative rotations. The use of the acid and 0.5N NaOH solution as extraction agents resulted in a decrease in their specific rotations when compared respectively to the water and the 0.15N NaOH extract. For the basic extracts, the use of the more basic solution apparently results in a rupture of the chemical bonds responsible for the optical activity in the less basic extract. The positive rotations obtained for the less basic extract may be caused by the nucleic acids or their derivatives. (It is planned that both RNA and DNA, as well as their mononucleotides, will be treated with 0.5N NaOH to determine what effect this base has on the dispersion curves.

The reason for the acid-soluble extract having a lower specific rotation than the water-soluble extract will also be explored. Since both these extracts have negative specific rotations, the possibility that proteins or their hydrolysis products are the cause of these negative rotations will be investigated.

The adaptation of the Cary spectrophotometer for these measurements has given rise to an instrumentation problem. This is the drifting of the base line due to the electronic components associated with the automatic slit control and readout. It is hoped that this problem may be overcome by obtaining the output of the sample and reference phototubes directly.

7.0 PROGRAM FOR NEXT PERIOD

The work performed during the past report period was directed toward two objectives: (1) validation of the theoretical conclusions reported in the previous period, that is, the use of the electronic ratio described as the "difference to a constant" and the simplified ratio of the attenuated energy of a single polarized beam divided by the unattenuated polarized beam; and (2) development of practical systems and processes for the rapid and efficient extraction of optically active substances from soil.

Both of these objectives were achieved; the minimum information required for establishing the feasibility of this approach to detection of extraterrestrial optical activity has been obtained. Effective boundary conditions, however, must be established before firm engineering design goals can be defined. Work for the next reporting period will be directed toward the quantitative explication of the processes outlined during the present work period. Specifically, the optical rotatory dispersion spectra of pure RNA, DNA, etc. subjected to the various extraction procedures described in this report will be determined. Additional extraction processes will be considered, along with optical rotatory spectra of some other complex biological materials which may be considered generally characteristic of life, e.g., chlorophyll.

8.0 APPENDIX

Theoretical Development of Modified Form of Ratio of Difference to a Constant

It may be shown that a linear relationship exists between the modified form of the ratio of the difference to a constant and the angular rotation of an optically active substance under specific conditions. By using a double-beam instrument in which one beam contains both a polarizer and analyzer prism and the other beam contains only a polarizer prism, this ratio may be defined by equation (1)

$$R = k_s/k_o = \frac{E'}{E_c'} \bigg/ \frac{E}{E_c} \quad (1)$$

where:

E = the intensity of a light beam as seen by a photomultiplier tube in the absence of an optically active substance. This beam contains an analyzer prism where its optical axis is set at either an angle of $+\theta$ or $-\theta$ with respect to the optical axis of the polarizer.

E_c = the intensity of a light beam as seen by a photomultiplier tube in the absence of an optically active substances and an analyzer prism.

E' and E_c' = the intensity as seen by the photomultiplier tubes in the presence of an optically active substance at the same settings under which E and E_c were measured.

Mathematically, E may be represented by equation (2)

$$E = A^2 \cos^2 \theta \quad (2)$$

where:

A represents the amplitude of the plane polarized beam and

$\cos \theta$ represents the projection of the plane-polarized beam on the optical axis of the analyzer prism.

E_c may be represented by equation (3)

$$E_c = A^2. \quad (3)$$

To obtain optimum sensitivity in the range where the photomultiplier tubes operate with essentially the same response, a neutral density filter with transmission, τ , may be placed in the beam which does not contain the analyzer prism.

Equation (3) may now be written as

$$E_c = A^2 \tau. \quad (3')$$

E_c can be set equal to E :

$$E = A^2 \cos^2 \theta = E_c = A^2 \tau$$

therefore:

$$\tau = \cos^2 \theta.$$

If an optically active substance which exhibits absorption, scattering, and circular dichroism is placed in both beams, equation (2) may be rewritten as equations (4a) or (4b), depending upon whether the substance is dextrorotatory or levorotatory.

$$E' = \frac{1}{4} A^2 e^{-k l c} e^{-k l c} \left(e^{\frac{-k_R l c}{2}} + e^{\frac{-k_L l c}{2}} \right)^2 \cos^2 (\theta - \alpha) \\ + \frac{1}{4} A^2 e^{-k l c} e^{-k l c} \left(e^{\frac{-k_R l c}{2}} - e^{\frac{-k_L l c}{2}} \right)^2 \sin^2 (\theta - \alpha) + \Sigma \quad (4a)$$

$$E' = \frac{1}{4} A^2 e^{-k l c} e^{-k l c} \left(e^{\frac{-k_R l c}{2}} + e^{\frac{-k_L l c}{2}} \right)^2 \cos^2 (\theta + \alpha)$$

$$+ \frac{1}{4} A^2 e^{-k\ell c} e^{-k_{\epsilon}\ell c} \left(e^{-\frac{k_R\ell c}{2}} - e^{-\frac{k_L\ell c}{2}} \right)^2 \sin^2 (\theta + \alpha) + \Sigma \quad (4b)$$

where:

Σ = amount of unpolarized light passing through the analyzer since scattering results in depolarization of plane polarized light.

α = angular rotation produced by the solution

k = absorption coefficient associated with the adsorption of right and left circularly polarized light by a chromophore.

k_R, k_L = absorption coefficients associated with the absorption of right and left circularly polarized light because of the interaction of an asymmetric environment with a chromophore.

k_{ϵ} = scattering coefficient

ℓ = path length

c = concentration of optically active solution

Under the same conditions, equation (3') may be rewritten as equation (5) in which $\tau = \cos^2 \theta$.

$$E_c' = \frac{1}{2} A^2 e^{-k\ell c} e^{-k_{\epsilon} c} \left(e^{-\frac{k_R\ell c}{2}} + e^{-\frac{k_L\ell c}{2}} \right) \cos^2 \theta \quad (5)$$

Substitution of equations (2), (3'), (4a), and (5) into equation (1), cancellation of the common terms in both the numerators and demoninators, and taking advantage of the trigonometric identities:

$$\cos^2 x + \sin^2 x = 1$$

$$\cos^2 x - \sin^2 x = \cos 2x$$

leads to equation (6).

$$R = \frac{k_s}{k_o} = \frac{1}{2\cos^2\theta} + \frac{e^{-\frac{k_L \ell c}{2}} e^{-\frac{k_R \ell c}{2}} \cos 2(\theta - \alpha)}{\left[e^{-\frac{k_L \ell c}{2}} e^{-\frac{k_R \ell c}{2}} \right] \cos^2\theta} \quad (6)$$

The Σ term in equation (4a) may be neglected in this treatment because this quantity is small compared to the total intensity, as seen by the photomultiplier tube.

Substituting the trigonometric relationships:

$$2\cos^2\theta = 1 + \cos 2\theta$$

$$\cos 2\theta = \frac{1-t^2}{1+t^2} \quad \text{and} \quad \sin 2\theta = \frac{2t}{1+t^2}$$

where:

$$t = \tan \theta$$

into equation (6) and simplifying, results in equation (7)

$$R = \frac{k_s}{k_o} = \frac{1+t^2}{2} + \frac{e^{-\frac{k_L \ell c}{2}} e^{-\frac{k_R \ell c}{2}}}{e^{-\frac{k_L \ell c}{2}} + e^{-\frac{k_R \ell c}{2}}} \left[(1-t^2)\cos 2\alpha + 2t \sin 2\alpha \right] \quad (7)$$

If we set θ equal to 45° , $\tan \theta = 1$. Substituting $t=1$ into equation (7) yields equation (8)

$$R = \frac{k_s}{k_o} = 1 + \frac{e^{-\frac{k_L \ell c}{2}} e^{-\frac{k_R \ell c}{2}}}{e^{-\frac{k_L \ell c}{2}} + e^{-\frac{k_R \ell c}{2}}} 2 \sin 2\alpha. \quad (8)$$

By allowing $\theta = 45^\circ$, the $(1-t^2)\cos 2\alpha$ term in equation (7) becomes equal to 0.

Multiplying both the numerator and demoninator of equation 8 by

$$\frac{e^{\frac{k_R \ell c}{2}}}{e^{\frac{k_R \ell c}{2}}} \quad \frac{e^{\frac{k_L \ell c}{2}}}{e^{\frac{k_L \ell c}{2}}}$$

leads to equation (9)

$$R = \frac{k_s}{k_o} = 1 + \frac{e^{\frac{(k_L - k_R)\ell c}{2}}}{e^{\frac{(k_L - k_R)\ell c}{2}} + 1} (2 \sin 2\alpha). \quad (9)$$

If we let $x = \frac{\Delta k \ell c}{2} = \frac{(k_L - k_R)\ell c}{2}$, equation (9) may be written as

$$R = \frac{k_s}{k_o} = 1 + \frac{e^x}{e^{2x} + 1} (2 \sin 2\alpha). \quad (10)$$

Both $\frac{e^x}{e^{2x} + 1}$ and $\sin 2\alpha$ can be expanded in a power series around zero.

$$\frac{e^x}{e^{2x} + 1} = \frac{1}{2} \left[1 - \frac{x^2}{2!} + \frac{5x^4}{4!} - \dots \right] \quad (11)$$

$$\sin 2\alpha = 2\alpha \left[1 - \frac{(2\alpha)^2}{3!} + \frac{(2\alpha)^4}{5!} - \dots \right] \quad (12)$$

It can be readily shown that the series represented in equations (11) and (12) converge for all values of x and α . In addition, the series represented by equation (12) converges rapidly when $-1 < \alpha < 1$. If R is plotted as a function of α , it should be symmetrical with respect to the α axis because only even-power terms existed in the series represented by equation (12). The series represented by equation (11) is independent of the sign of x because only even powers of x exist in this series, and it also converges rapidly $-1 < x < 1$. Substitution of equations (11) and (12) into equation (10) and combining terms results in equation (13).

$$R = \frac{k_s}{k_o} = 1 + 2\alpha \left[1 - \frac{(\Delta k l_c)^2}{8} \right] - \left[1 - \frac{(\Delta k l_c)^2}{8} \right] \left[\frac{(2\alpha)^3}{3!} - \frac{(2\alpha)^5}{5!} \right] + \left[\frac{5(\Delta k l_c)^4}{16 \cdot 4!} \right] \left[2\alpha - \frac{(2\alpha)^3}{3!} + \frac{(2\alpha)^5}{5!} \right] \quad (13)$$

For angular rotations less than 6° , equation (13) reduces to equation (14).

$$R = \frac{k_s}{k_o} = 1 + 2\alpha \left[1 - \frac{(\Delta k l_c)^2}{8} \right] \quad (14)$$

If it is considered that the intensities of both beams in the polarimeter may differ, equation (3') can be rewritten as

$$E_c = B^2 \tau \quad (15)$$

where:

B = amplitude of plane-polarized beam in the channel which contains the neutral density filter.

Since $E_c \neq E$ in equation (2), it can be assumed that $\tau \neq \cos^2 \theta$; therefore, in the presence of an optically active substance, equation (5) is modified to equation (16)

$$E_c^{-1} = \frac{1}{2} B^2 e^{-k l_c} e^{-k l_c} (e^{-k_R l_c} + e^{-k_L l_c}) \tau \quad (16)$$

Substitution of equations (15), (16), (2), and (14a) into equation (1), and again neglecting the ϵ term, leads to equation (17).

$$\begin{aligned}
R = \frac{k_s}{k_o} &= \frac{1}{4} A^2 e^{-k_R l c} e^{-k_L l c} \left(e^{\frac{-k_R l c}{2}} + e^{\frac{-k_L l c}{2}} \right)^2 \cos^2 (\theta - \alpha) \\
&+ \frac{1}{4} A^2 e^{-k_R l c} e^{-k_L l c} \left(e^{\frac{-k_R l c}{2}} + e^{\frac{-k_L l c}{2}} \right)^2 \sin^2 (\theta - \alpha) \\
&\dots\dots\dots \\
&\frac{1}{2} B^2 e^{-k_R l c} e^{-k_L l c} (e^{-k_R l c} + e^{-k_L l c}) \tau \\
&= \frac{A^2 \cos^2 \theta}{B^2 \tau} \quad (17)
\end{aligned}$$

Cancellation of the common terms in both the numerator and denominator, and taking advantage of the trigonometric identities:

$$\begin{aligned}
\cos^2 x + \sin^2 x &= 1 \\
\cos^2 x - \sin^2 x &= \cos 2x
\end{aligned}$$

equation (17) may be rewritten as equation (18).

$$R = \frac{k_s}{k_o} = \frac{1}{2 \cos^2 \theta} + \frac{e^{-k_L l c} - e^{-k_R l c} \cos 2(\theta - \alpha)}{[e^{-k_L l c} + e^{-k_R l c}] \cos^2 \theta} \quad (18)$$

Equation (18) is the same as equation (6); therefore, the ratio, R, is still related to angular rotation by equation (14).